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Behavioral Responses of Crustacean Larvae to Rates of Salinity Change

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Abstract. The ontogeny of behavioral responses of larvae of the crabs *Rhithropanopeus harrisii* and *Neopanope sayi* to rates of change in salinity were analyzed with a video system. A salinity increase evoked an ascent in both species. For *R. harrisii* the threshold rate of increase was 1.1×10^{-3} ppt s^{-1} for the first and last zoeal stages and changed little with acclimation salinity. *N. sayi* larvae were more sensitive, as thresholds were 2.8×10^{-4} ppt s^{-1} for Stage I zoeae and 7.0×10^{-4} ppt s^{-1} for Stage IV. This difference in sensitivity may relate to the magnitude of salinity gradients in the estuarine/coastal areas inhabited by the larvae. At threshold rates of salinity increase the absolute amount of change before a response was lower for Stage I zoeae (0.09–0.11 ppt) than Stage IV zoeae (0.21–0.29 ppt) for both species. Decreases in salinity did not induce the expected descent response in either species at rates up to 4.7×10^{-2} ppt s^{-1}. The different responses in a salinity gradient may have resulted because the rate threshold and absolute amount of change before a response to a salinity increase were below those for a salinity decrease. Considering larval sinking rates and normal environmental salinity gradients, larvae of both species can respond to rates and amounts of salinity increase in their environment. The ascent response may be important for keeping larvae up in the water column and reducing the likelihood that they will encounter the bottom.

Introduction

A change in salinity dramatically modifies the behavior of many species of crustacean larva (Sulkin, 1984; Forward, 1987). Considering those cases where responses are reported, there is a characteristic pattern of responsiveness. A decrease in salinity, as occurs high in a stratified water column, induces downward movement (Lance, 1962; Scarratt and Raine, 1967; Harder, 1968; Hughes, 1969; Roberts, 1971). The underlying responses include positive geotaxis (Latz and Forward, 1977; Sulkin *et al.*, 1980), passive sinking (Latz and Forward, 1977), and negative phototaxis (Lyon, 1906; Edmondson and Ingram, 1939; Latz and Forward, 1977). Increases in salinity usually occur low in the water column and generally evoke upward movement. This ascent can result from negative geotaxis (Latz and Forward, 1977; Sulkin *et al.*, 1980), an increase in swimming speed (Sulkin *et al.*, 1980), and positive phototaxis (Latz and Forward, 1977; Sulkin and Van Heukelem, 1982).

Thus past studies indicate that larvae have a negative feedback system in which salinity conditions high in the water column induce downward movement and those low in the water column induce the opposite responses. This system could function for depth maintenance according to salinity. However, for this to occur, larvae must have sufficient sensitivity to detect the actual salinity changes that occur in their environment.

The minimum step change in salinity (threshold) necessary to induce behavioral responses varies with species. The threshold for a reversal in phototactic sign for larvae of the crab *Rhithropanopeus harrisii* upon step decreases in salinity ranges from 1.1 to 2.0 ppt (Latz and Forward, 1977). Measurements in haloclines indicate that some zoeal stages of *Callinectes sapidus* (Sulkin and Van Heukelem, 1982) and *Pagurus longicarpus* (Roberts, 1971) can detect salinity discontinuities at least as low as 2.5 ppt, while *Uca pugnax* requires a change of 6 ppt (O'Connor and Epifanio, 1985).

The problem with these past studies is that larvae were exposed to step changes in salinity or sharp haloclines.

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In the field larvae encounter vertical gradients in salinity and less commonly experience sharp haloclines. Thus in the water column larvae perceive changes in salinity that depend upon the magnitude of the vertical gradient and rates of vertical movement.

The present study was undertaken (1) to determine the lowest rates of salinity change that evoke behavioral responses, (2) to compare these rates to those that a larva could encounter in the water column, and (3) to determine the absolute amount of salinity change that must occur before a response. The study compared larvae of the crabs *Rhithropanopeus harrisii* and *Neopanope sayi*. These species were used because both live as adults in estuaries but the behavior of *R. harrisii* larvae results in retention in upper estuarine areas (Cronin, 1982), while *N. sayi* larvae are not retained and undergo development in lower estuarine and coastal areas (Sandifer, 1975; Dittel and Epifanio, 1982; Salmon *et al.*, 1986). Thus each larval species is potentially exposed to a different salinity regime, since salinity gradients are greater in estuarine than coastal areas.

Materials and Methods

Ovigerous *Rhithropanopeus harrisii* were collected from the Neuse River estuary (North Carolina) from May to August, 1988. Crabs were placed in either 10 or 20 ppt seawater (acclimation salinity), which was passed through a 5-μ filter. Since the females adjust their body fluid osmolality to salinity changes in 8 h (Diamond *et al.*, 1989), it was assumed that the embryos were similarly adjusted at the time of hatching, which occurred after 8 h in the acclimation salinities. Groups of larvae were reared in both salinities and experiments conducted with Stages I and IV zoeae. Even though the upper salinity (20 ppt) is optimal for developmental success, larvae can complete development in 10 ppt (Costlow *et al.*, 1966).

Ovigerous *Neopanope sayi* (Smith) were collected near the entrance to the Newport River estuary (North Carolina) from June to September, 1988, and females placed in 28 ppt seawater (acclimation salinity). Larvae were reared at this salinity and Stages I and IV zoeae used in the experiments. This salinity was selected because it is close to the salinities where maximum larval abundance occurs in the field (Sandifer, 1975) and to permit the experimental salinity changes to encompass normal salinities encountered by the larvae. Responses after acclimation to a lower salinity were not tested because there was almost no change in sensitivity by *R. harrisii* larvae when acclimated to different salinities. Stages I and IV zoeae were tested for an ontogenetic change in responsiveness because each species has four zoeal stages.

Rearing took place in a controlled environmental chamber (Sherer, Model CEL4-4) on a 14:10 LD cycle and 25°C. Throughout development, larvae were transferred daily to clean seawater and fed newly hatched *Artemia* spp. nauplii. All experiments were performed in the 6-h interval in the middle of the light phase to avoid complications due to biological rhythms in behavior. Larvae were light-adapted to room fluorescent light (intensity = about 1 W m^{-2}) prior to all experiments. In most cases a minimum of five groups of larvae, each from a separate female, were tested in each experiment.

Experimental approach

Larval responses to different rates of salinity change were measured in a chamber having three vertical cylindrical sections (section height = 2.5 cm; diameter = 2.5 cm; Fig. 1). For a salinity decrease, low salinity water was added to the upper section and mixed by a slowly rotating stirring paddle. Larvae were confined to the middle section by plankton netting (75 μ mesh) at the upper and lower boundaries, and their behavior monitored and recorded with a closed circuit television system. For viewing, animals were illuminated with far-red light (maximum transmission 775 nm), to which larvae are not responsive (Forward and Cronin, 1979). The lower section was used for salinity increases. High salinity water was added and mixed with a magnetic stirring bar. Preliminary measurements of larval swimming indicated slow stirring in the upper and lower sections had no apparent effect on movement.

To insure that there were constant amounts of water in all sections and constant flow rates through the center section, the different salinities were delivered to the appropriate end section by a variable speed peristaltic pump, and water was extracted at the same rate from the other end section by the same pump. For example, to induce a salinity decrease, low salinity water was pumped into the upper chamber, while water was removed from the lower chamber at the same rate. Dye studies indicated the flow of water through the netting into the center section was approximately laminar.

The rates of change in salinity were varied through differences between input and acclimation salinity and pumping rates. The actual salinity in the center of the larval section was measured with a conductivity probe (Model PP1042; Radiometer), which was calibrated in the center section with water of known salinity. The smallest detectable change in salinity was about 0.09 ppt. Water temperature remained relatively constant at 25°C during calibration and throughout the experiments. The digital readout of conductivity was viewed by a second video camera and inserted in the video picture with a video screen splitter (Model U2705P; Vicon Industries, Inc.). A record of time was also inserted in the picture by

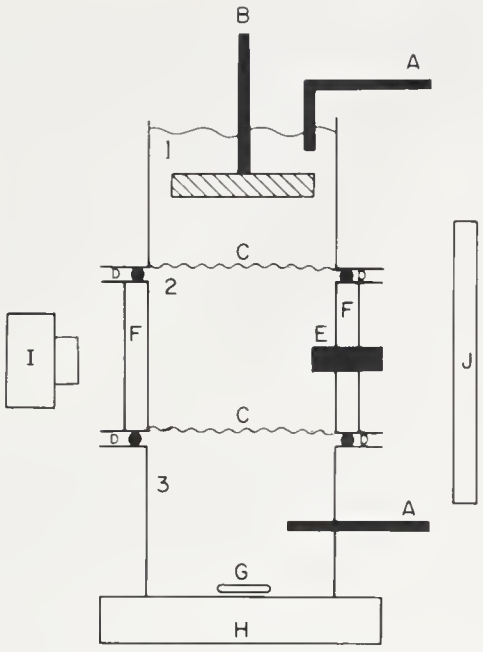

Figure 1. Horizontal view of the test chamber consisting of equal size cylindrical upper (1), test (2), and lower (3) sections (not drawn to scale). A—fluid input/output tube connected to the peristaltic pump; B—stirring paddle connected to a variable speed stirring motor; C—75 μ mesh plankton netting; D—O-ring; E—conductivity probe connected to meter with digital readout; F—rectangular water-filled chamber surrounding the test chamber; G—magnetic stirring bar; H—magnetic stirrer; I—video camera; J—far-red illumination light. In the actual chamber the video camera and conductivity probe are oriented perpendicular to each other.

a Field/Frame Counter (QSI Systems, Inc.). In this way larval behavior, conductivity, and time were recorded simultaneously on video tape. The actual rates of change in salinity were calculated from the measurements of time and conductivity. In each experiment the rate of change in salinity quickly increased up to a maximal rate for each flow rate/salinity difference condition and then remained approximately uniform through the time when responses were measured.

Experimental procedures

The same general procedure tested for responses to salinity increases and decreases. A group of approximately 75 Stage I or 25 Stage IV zoeae was placed in the test chamber and after 1 min in darkness the peristaltic pump and videotape recorded were started. The experiment continued until at least 1 ppt change occurred upon a salinity increase and 5 ppt change upon a decrease. Any response was evident after this amount of salinity change. Larvae were then removed, the chamber rinsed with acclimation salinity water, and a new group of larvae placed in the chamber. Larvae were only tested once at each developmental stage. To control for responses to flow through the chamber, larvae were tested using the above procedure at the maximum test flow rate with acclimation salinity water.

Analysis

All experiments were conducted with the test chamber in a light-tight space. Thus larvae were only illuminated with far-red light. Since they were functionally in darkness in this situation, the possible behavioral responses to changes in salinity were changes in activity and/or geotaxis.

To analyze for behavioral responses, the larval section of the test chamber was divided into three equal horizontal subsections by a template placed over the video screen. The number of larvae in each subsection was counted before (control) and after a change in salinity, and the percentage of larvae in each subsection was calculated. When testing different rates of salinity change, responses were measured after a 0.75 ppt change for a salinity increase and a 5.0 ppt change for a salinity decrease. These absolute changes were used because responses were clearly evident (Fig. 6) for a salinity increase of 0.75 ppt and a 5.0 ppt decrease is about 3-fold larger than the threshold for step decreases for *R. harrisii* larvae (Latz and Forward, 1977). Thus responses to salinity decreases should have been evident by this amount of change.

Ascent and descent responses were expected upon a salinity increase and decrease, respectively. Thus the change in the percentage of larvae in the upper section was monitored upon a salinity increase and in the lower section upon a salinity decrease. Arcsine transformed data were used for statistical tests and to calculate means and standard errors. Back transformed means and standard errors are plotted in the figures. If paired observations were made before (control) and upon stimulation (experimental) of each group of animals, a t-test for paired comparisons was used to test for differences. In cases where a control was compared to responses at different times after the beginning of stimulation, then the Dunnett's t-test for multiple comparisons with a control was used to test for significant differences ($P < 0.05$; Dunnett 1964).

Results

Salinity increase

Larvae were extremely sensitive to a salinity increase (Fig. 2A). These responses were not due to fluid flow through the chamber, as significant changes in larvae dis-

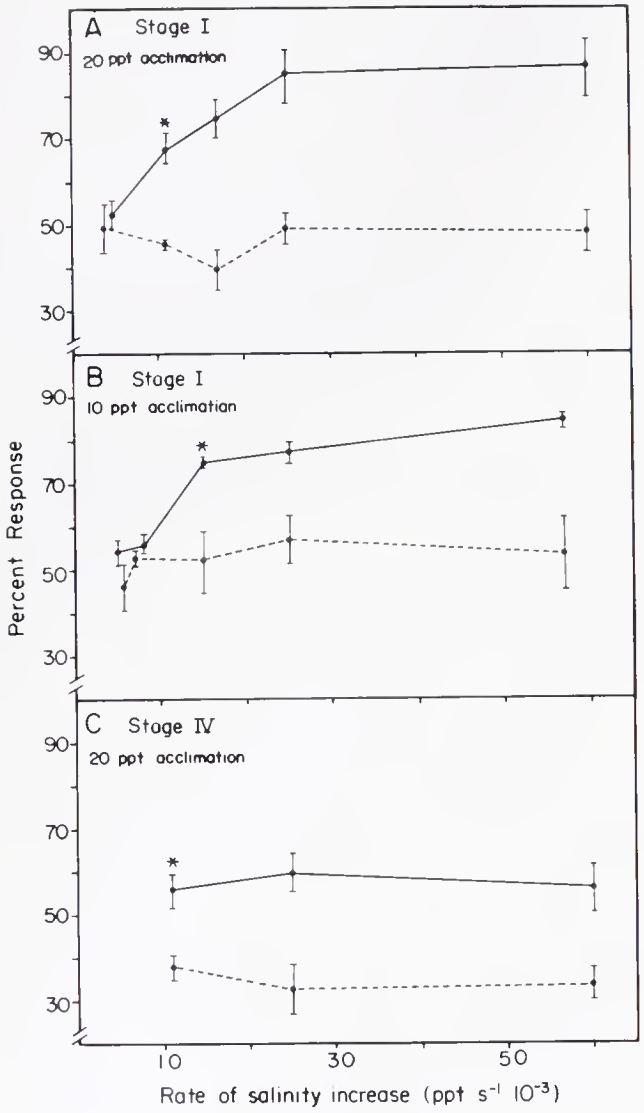

Figure 2. The percentage of *Rhithropanopeus harrisii* larvae aggregating in the upper section of the test chamber before stimulation (control—dashed line) and after 0.75 ppt salinity increase (experimental—solid line) for Stage I zoeae acclimated to 20 ppt (A), and 10 ppt (B), and Stage IV zoeae acclimated to 20 ppt (C). The average number of determinations for each rate was 5 and means and standard errors are plotted. The asterisks indicate the slowest rate (threshold) to induce a response that was significantly ($P < 0.05$) greater than the control.

tributions did not occur under conditions of flow alone (no salinity change) at the fastest test flow rate (Table I).

For *R. harrisii* Stage I zoeae the lowest rates of salinity increase (threshold) to induce a significant ascent response upon acclimation to 20 ppt (Fig. 2A) and 10 ppt (Fig. 2B) were 1.1×10^{-3} and 1.5×10^{-3} ppt s^{-1}, respectively. Since these thresholds are similar and in both cases responses were measured after the same absolute amount of salinity change (0.75 ppt), the larvae responded to either the absolute amount or rate of change in salinity and not the absolute levels of salinity. Stage IV zoeae (Fig. 2C) had similar sensitivity, as they displayed a significant response at the lowest test rate (1.1×10^{-3} ppt s^{-1}). Since this rate is either at or slightly above the threshold, it will be used as the threshold in future discussions.

N. sayi larvae were more sensitive to a salinity increase than *R. harrisii* larvae. Stage I zoeae of *N. sayi* responded to the lowest test rate (2.8×10^{-4} ppt s^{-1}; Fig. 3a), whereas Stage IV were less sensitive with a threshold at 7.0×10^{-4} ppt s^{-1} (Fig. 3b).

Salinity decrease

Both species of larvae were unresponsive to salinity decreases. The responses were unaffected by flow from the top to the bottom of the test chamber, as the distributions of larvae in the lower section did not change significantly upon exposure to the fastest test rates of flow alone (Table I).

Responses of *R. harrisii* larvae after a 5-ppt change in salinity are shown in Figure 4. Upon acclimation to 20 ppt, Stage I zoeae did not show a significant descent re-

Table I

Control for flow

Condition	Flow rate (ml/min)	Control m (%)	Control SD	Experimental m (%)	Experimental SD
Salinity increase					
Rhithropanopeus harrisii					
Stage I—20 ppt	7.2	46.0	6.1	39.7	6.7
Stage I—10 ppt	4.1	44.7	5.6	43.7	4.2
Stage IV—20 ppt	4.1	35.2	7.9	33.5	7.0
Neopanope sayi					
Stage I—28 ppt	4.1	41.3	11.2	48.5	4.1
Stage IV—28 ppt	1.94	38.3	10.1	42.8	15.3
Salinity decrease					
R. harrisii					
Stage I—20 ppt	4.1	35.3	4.9	39.4	7.2
Stage IV—20 ppt	7.2	42.3	4.5	52.9	9.9
N. sayi					
Stage I—28 ppt	4.1	38.8	4.3	42.4	2.6
Stage IV—28 ppt	4.1	52.4	6.5	40.6	10.1

The percentage of larvae in the upper section upon a simulated salinity increase and in the lower section with a simulated salinity decrease. Responses are shown before experimentation (control) and after exposure to the maximum experimental flow rate (experimental) of acclimation salinity water through the test chamber. Experimental values were measured at the same time after the beginning of flow as measurements were made upon a real salinity change. The number of groups of larvae tested was 5 in all cases and means (m) and standard deviation (SD) are shown for arcsine transformed data. In no case was there a significant difference between the experimental and control means.

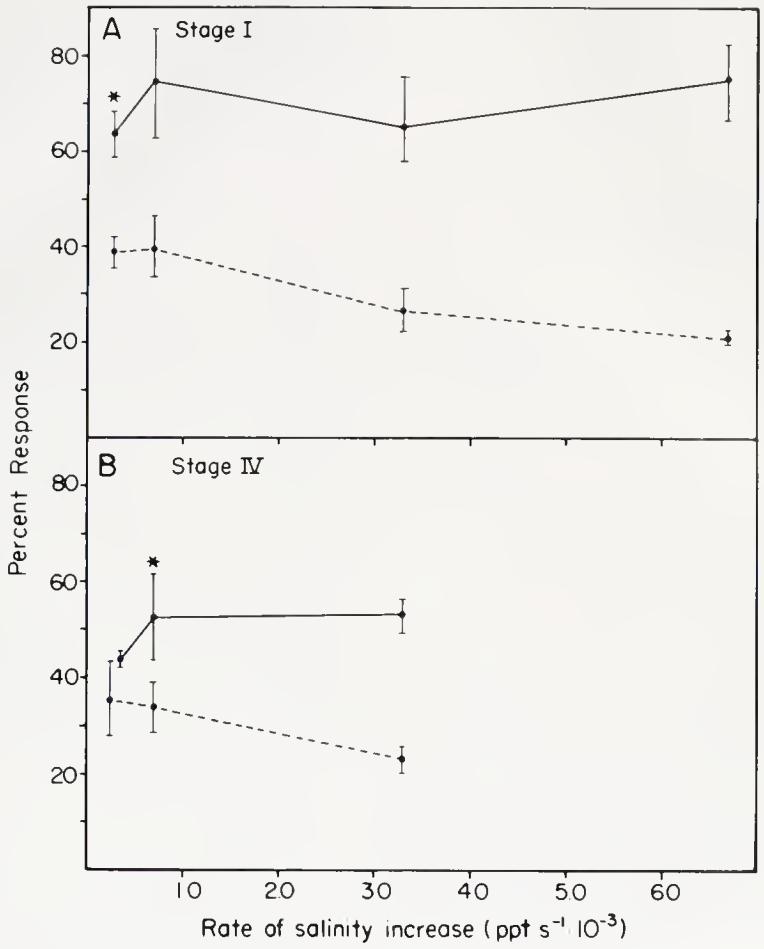

Figure 3. The percentage of *Neopanope sayi* larvae aggregating in the upper section of the test chamber before stimulation (Control—dashed line) and after 0.75 ppt salinity increase (Experimental—solid line) for Stages I (A) and IV (B) zoeae acclimated to 28 ppt. Five determinations were made for each rate, and means and standard errors are plotted. The asterisks indicate the slowest rate (threshold) that induced a significant ($P < 0.05$) response.

sponse (Fig. 4A) at test rates up to the fastest rate possible with available equipment (4.1×10^{-2} ppt s^{-1}). The test rates ranged from those used in the salinity increase experiments (Fig. 2) to an order of magnitude faster. When acclimated to 10 ppt, Stage I zoeae also failed to respond (transformed values, control m = 39.5%, SD = 5.2; experimental m = 42.7%, SD = 1.9; n = 5) at the fastest possible test rate (2.8×10^{-3} ppt s^{-1}). Stage IV zoeae were similarly unresponsive (Fig. 4B).

Responses of *N. sayi* larvae are also shown after a 5-ppt decrease in salinity (Fig. 5). Responses of Stages I and IV zoeae were not significantly greater than control levels. Thus *N. sayi* larvae were unresponsive to salinity decreases up to the fastest test rates (Stage I, 3.5×10^{-2} ppt s^{-1}; Stage IV, 4.7×10^{-2} ppt s^{-1}).

Absolute salinity increase

Responses upon absolute amounts of salinity increase were measured at threshold rates of change. For *R. harrisii*, different amounts of salinity change were required for different zoeal stages, as a significant response occurred after 0.09 ppt change for Stage I zoeae and 0.29 ppt for Stage IV zoeae (Fig. 6A, B). The decrease in sensitivity with later zoeal stages was also observed for *N. sayi* larvae. Stage I zoeae responded after 0.11 ppt change while Stage IV zoeae required 0.21 ppt change (Fig. 6C, D).

Discussion

The expected responses of zooplankton to step salinity changes are an ascent upon a salinity increase and descent upon a salinity decrease (Forward, 1976). In the present experiments with salinity gradients, crab larvae displayed the ascent response but the descent response was absent. Since all experiments were conducted in darkness, the ascent resulted from an increase in swimming speed or negative geotaxis (Forward, 1988).

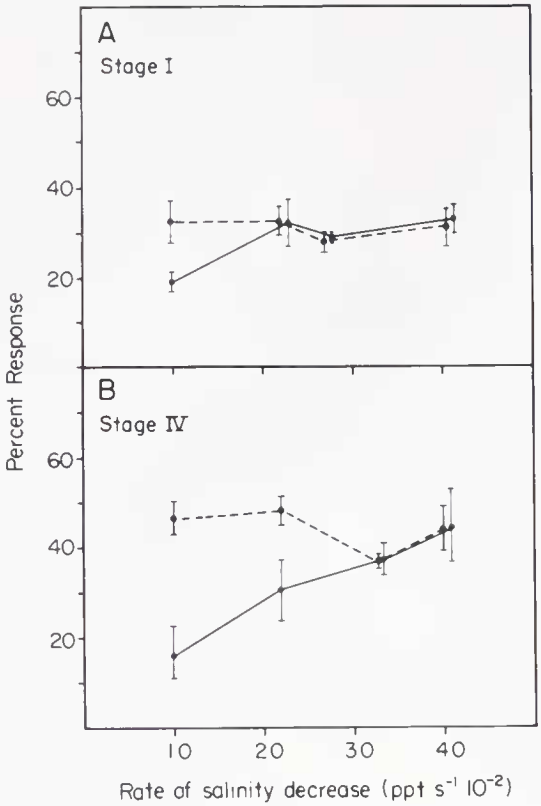

Figure 4. The percentage of *Rhithropanopeus harrisii* larvae aggregating in the lower section of the test chamber before stimulation (Control—dashed line) and after 5.0 ppt salinity change (Experimental—solid line) for Stages I (A) and IV (B). Zoeae were acclimated to 20 ppt. Five determinations were made for each rate, and means and standard errors are plotted.

The test species were selected because *R. harrisii* larvae undergo development in upper estuarine areas (Cronin, 1982), while *N. sayi* larvae develop in lower estuarine and coastal areas (Sandifer, 1975; Dittel and Epifanio, 1982; Salmon *et al.*, 1986). The lowest rate of salinity increase to evoke an ascent response by *R. harrisii* (1.1×10^{-3} ppt s^{-1}) was similar throughout zoeal development and changed little with acclimation salinity. Thus larvae respond to the change in salinity not to an absolute salinity. These results are similar to those found using step changes in salinity (Latz and Forward, 1977). Stages I and IV zoeae of *N. sayi* showed the same behavioral responses but the thresholds (Stage I, 2.8×10^{-4} ppt s^{-1}; Stage IV, 7.0×10^{-4} ppt s^{-1}) were much lower than those for *R. harrisii* larvae. This increased sensitivity by *N. sayi* larvae may result from the environmental salinities zoeae encounter, since larger gradients exist in upper estuarine areas (*e.g.*, Cronin, 1982) than lower estuarine/coastal areas (*e.g.*, Williams *et al.*, 1967).

For some species there are ontogenetic changes in responses to salinity. In the present study Stages I and IV zoeae of both species displayed small differences in sensitivity to a salinity increase. The minimum salinity sensitivity is characterized by the threshold rate of change and the absolute amount of change before a response. The thresholds were the same for both first and last zoeal stages of *R. harrisii*, but for *N. sayi*, Stage I zoeae were 2.5 times more sensitive than Stage IV zoeae. At threshold rates of salinity increase, the absolute amount of change before an ascent response was always less for Stage I than Stage IV zoeae of both species. Thus older larvae appear to be less sensitive to a salinity increase.

None of the test larvae showed descent responses to a salinity decrease in a gradient. The absence of a descent response is unexpected because it has been observed in other species upon step changes in salinity (Lance, 1962; Scarratt and Raine, 1967; Harder, 1968; Hughes, 1969; Roberts, 1971), and a positive geotaxis clearly occurs in Stages I and IV zoeae of *R. harrisii* upon step decreases in salinity (Latz and Forward, 1977). The reason for this discrepancy may be the difference between responses upon step changes and in gradients.

When larvae are tested with a step decrease in salinity, they are exposed to a constant change in salinity that is uniform throughout the test chamber. Thus when larvae

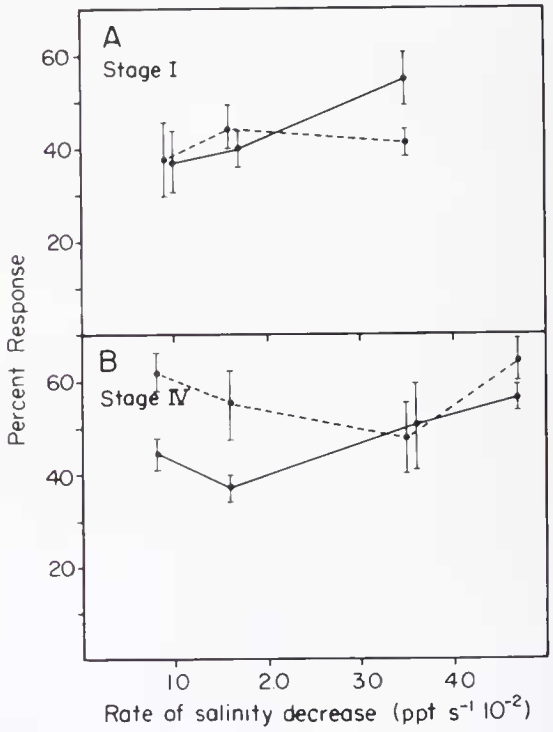

Figure 5. The percentage of *Neopanope sayi* larvae aggregating in the lower section of the test chamber before stimulation (Control—dashed line) and after 5.0 ppt salinity change (Experimental—solid line) for Stages I (A) and IV (B) zoeae acclimated to 28 ppt. Five determinations were made at each rate and means and standard errors are plotted.

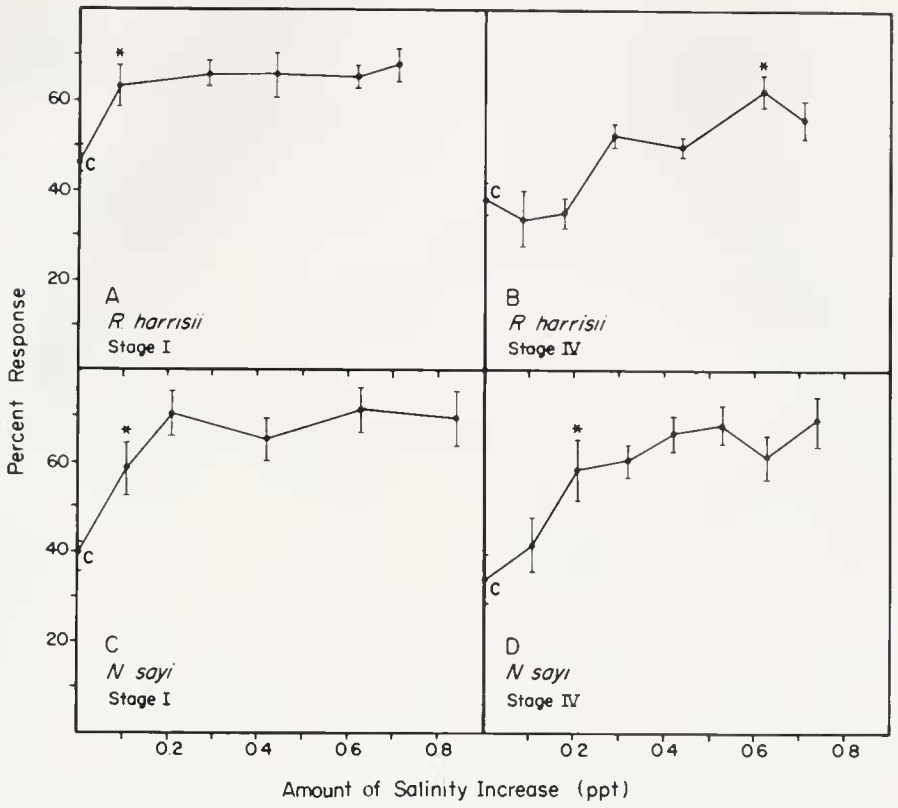

Figure 6. The percentage of *Rhithropanopeus harrisii* (A, B) and *Neopanope sayi* (C, D) larvae aggregating in the upper section upon exposure to the threshold rate of salinity increase (A, B = 1.1×10^{-3} ppt s^{-1}; C = 2.8×10^{-4} ppt s^{-1}; D = 7.0×10^{-4} ppt s^{-1}). Responses were measured after different absolute amounts of salinity change. *R. harrisii* larvae were acclimated to 20 ppt and *N. sayi* to 28 ppt. Five determinations were made for each zoeal stage except *R. harrisii* Stage IV zoeae where four determinations were made. Means and standard errors are plotted. An asterisk indicates the smallest salinity increase to induce a response significantly greater ($P < 0.05$) than the control (C) plotted at zero salinity change.

respond by ascending or descending, they are not exposed to a change in salinity during vertical movement. Similarly, when testing in sharp haloclines lower salinity water rests on top of higher salinity water. In this system larvae are either in one salinity or the other because the distance separating the salinities is small.

For the present experiments, there was a gradient of low to high salinity extending from the top of the experimental chamber to the bottom. In this situation larvae experience a continual increase in salinity upon descending and a continual decrease upon ascending. Thus if larvae respond to a salinity decrease and begin to descend, they will experience a salinity increase which evokes the opposite response. If there are differences in the threshold rates for responses to a salinity increase and decrease and the absolute amount of salinity change that must occur before a response, then only one directional response can occur in a gradient.

For example, the threshold rates for responses to a salinity increase are probably well below those to a salinity decrease for *R. harrisii* larvae. As for the absolute amount of change, responses of *R. harrisii* larvae to a salinity increase were clearly evident after 0.09 ppt for Stage I zoeae and 0.29 ppt change for Stage IV zoeae (Fig. 6). In contrast, Latz and Forward (1977) found that for both zoeal stages the threshold for step decreases in salinity ranged from 1 to 2 ppt. Thus *R. harrisii* larvae respond to both a slower rate of change and less absolute change upon a salinity increase in a gradient.

To explain the responses observed in the present experiments, one can assume that ascent and descent speeds of movement of *R. harrisii* larvae are similar (Latz and Forward, 1977). Now if the combination of larval upward movement and rate of salinity decrease in the test chamber becomes sufficient for a response to a salinity decrease, larvae will begin to descend. However, the rate of salinity increase experienced during a descent is well above threshold for a behavioral response and larvae move down until the absolute amount of salinity change (0.09–0.29 ppt) induces an ascent response. On

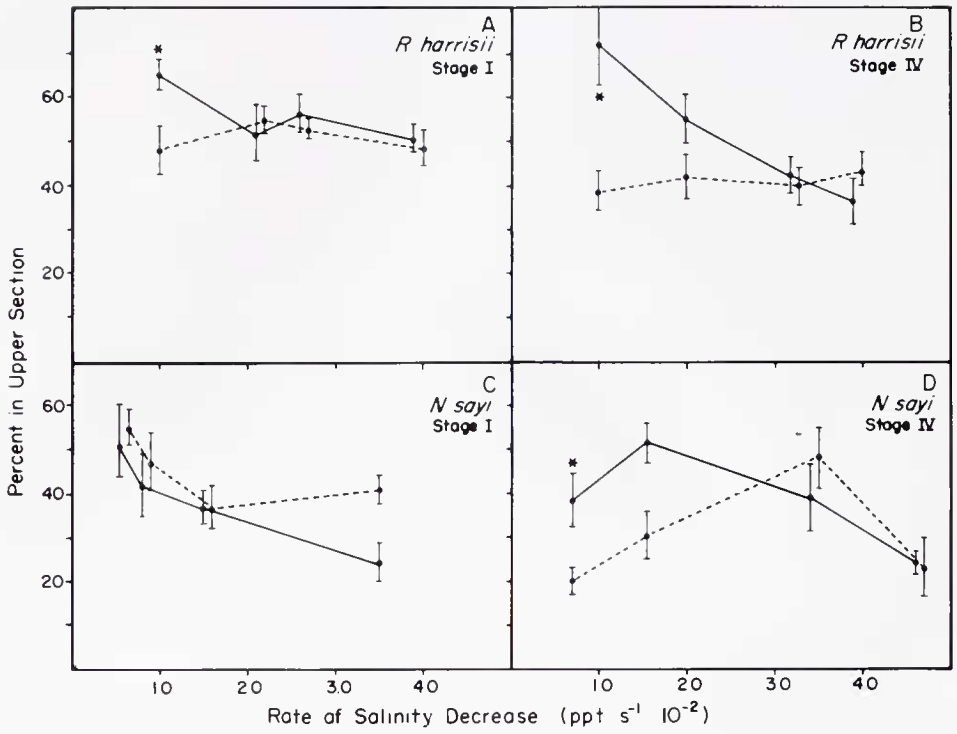

Figure 7. The percentage of *Rhithropanopeus harrisii* (A, B) and *Neopanope sayi* (C, D) zoeae aggregating in the upper section before stimulation (control—dashed line) and after 5.0 ppt change in salinity (experimental—solid line) at different rates of salinity decrease. *R. harrisii* larvae were acclimated to 20 ppt and *N. sayi* to 28 ppt. Five determinations were made for each rate and means and standard errors are plotted. An asterisk indicates rates at which the response is significantly ($P < 0.05$) greater than the control level.

ascending the rate of salinity decrease is sufficient to induce a behavioral response, but the absolute amount that salinity must change (1–2 ppt) before a response reversal is greater than upon descending (0.09–0.29 ppt). Thus larvae move up farther before the onset of a corrective response and should actually ascend upon a salinity decrease. This ascent response is evident in Figure 7 at the slowest rates of salinity decrease. At faster rates of salinity decrease, the salinity gradient in the experimental chamber became greater. Larvae display neither a descent (Figs. 4, 5) nor an ascent response (Fig. 7) and remained evenly distributed throughout the chamber. The absence of vertical movement occurred because the absolute vertical distances larvae moved during the descent and ascent responses probably became smaller in the steeper gradients.

Alternatively, upon a salinity increase, larvae respond to a slower rate of change than upon a salinity decrease. If exposed to a slow rate of salinity increase larvae will continually ascend in a gradient because the rate of salinity decrease encountered during the ascent is below threshold. The implications of these responses are that larvae should show responses to both increases and decreases in salinity at sharp haloclines as might occur after heavy rains, at fronts and in a two-layer estuary. Alternatively, larvae will ascend in small salinity gradients.

A consideration is whether crustacean larvae can respond to vertical salinity gradients in their environment. For *R. harrisii*, previous studies of responses to step changes in salinity (Latz and Forward, 1977) and of environmental salinity gradients (Cronin, 1982) suggest larvae should respond to environmental conditions. In contrast, a comparison of responses of *Callinectes sapidus* in haloclines with salinity levels in the larval habitat led Sulkin and Van Heukelem (1982) to conclude that the gradients are too small in nature to impede upward movement.

In the present study, responses to a salinity increase were most pronounced. For *R. harrisii* larvae, Cronin (1982) found a 1 to 8 ppt salinity difference between surface and bottom waters in a 3 to 4 m-deep water column at times of changing tidal flow direction in areas inhabited by the larvae. At other tidal phases, salinity was identical in surface and bottom waters. Assuming continuous salinity change from the surface to bottom, the predicted minimum and maximum salinity change ranges from

Table II

Calculation of the minimal detectable salinity change per m

Threshold rate salinity increase (ppt s^{-1})	Mean sinking rate (mm s^{-1})	Minimum detectable gradient of salinity increase (ppt m^{-1})
Rhithropanopeus harrisii		
Stage I—1.1×10^{-3}	3.1	0.35
Stage IV—1.1×10^{-3}	7.8	0.14
Neopanope sayi		
Stage I—2.8×10^{-4}	4.4	0.064
Stage IV—7.0×10^{-4}	6.2	0.11
Slowest test rate salinity decrease (ppt s^{-1})	Mean ascent rate (mm s^{-1})	Gradient of salinity decrease (ppt m^{-1})
R. harrisii		
Stage I—1.0×10^{-2}	6.3	1.6
Stage IV—1.0×10^{-2}	6.2	1.6
N. sayi		
Stage I—$.07 \times 10^{-2}$	3.2	2.2
Stage IV—$.07 \times 10^{-2}$	5.3	1.3

Rates are from Figures 2–5. Mean sinking (Latz and Forward, 1977) and ascent speeds (Forward and Wellins, 1988) for *R. harrisii* are at 20 ppt, while those for *N. sayi* are at 32 ppt (unpub. data). The minimal detection rate in ppt/m is calculated as (threshold rate/sinking rate)1000.

0.25 ppt m^{-1} to 2.7 ppt m^{-1}. A conservative measure of the speed of downward movement is larval sinking speed. If *R. harrisii* sink continuously, then the minimum salinity change they can detect is 0.35 ppt m^{-1} for Stage I zoeae and 0.14 ppt m^{-1} for Stage IV zoeae (Table II). Thus larvae can detect rates of salinity increase that are close to or slightly below the minimum rate of salinity change measured in their environment.

The minimum detectable salinity increases for *N. sayi* larvae are less than those for *R. harrisii* larvae (Table II) and range from 0.064 to 0.11 ppt m^{-1}. Measurements in lower estuarine (Williams *et al.*, 1967) and coastal areas of southeastern United States (Atkinson *et al.*, 1983; T. Johnson pers. comm. of salinity profile made from the R. V. Cape Hatteras along the North American east coast) suggest that sufficient vertical salinity gradients exist in areas inhabited by *N. sayi* larvae.

A response to a salinity increase requires not only a sufficient rate of increase but also an absolute amount of change. The minimum measured absolute amount ranged from 0.09 to 0.29 ppt for *R. harrisii* larvae and 0.11 to 0.21 ppt for *N. sayi* (Fig. 6). These amounts of change occur in areas inhabited by the larvae when gradients are present.

The minimum detectable salinity decrease gradient cannot be calculated because a descent response was not observed at any rate of salinity decrease. It is always possible that the foregoing explanation is incorrect and the absence of a response to a salinity decrease occurred because test rates were abnormally high. If larvae had responded to the slowest test rates of salinity decrease, then *R. harrisii* larvae could detect a gradient of 1.6 ppt m^{-1} and *N. sayi* gradient from 1.3 to 2.2 ppt m^{-1} (Table II). These values are within the levels observed in nature and thus are not abnormally high. The behavioral determination of detectable salinity decrease gradients may be impossible because of the extreme sensitivity to a salinity increase.

Considering the minimum detectable gradients of salinity increase, sinking rates (Table II), and the minimum absolute amount of detectable salinity increase, it is possible to calculate the descent time under these conditions before an ascent response occurs. It is assumed that larvae sink continuously. For *R. harrisii*, the times for Stages I and IV zoeae are 1.4 and 4.4 min, respectively, whereas the times for Stages I and IV zoeae of *N. sayi* are longer at 6.5 and 5.1 min, respectively. These times can be compared to rates of sensory adaptation to a change in salinity. Latz and Forward (1977) found that *R. harrisii* larvae showed total recovery to step changes in salinity in 5.5 min. Thus *R. harrisii* larvae can detect the minimum salinity gradients. If *N. sayi* larvae adapt at the same rates as *R. harrisii* larvae, then Stage IV zoeae of *N. sayi* can also detect the minimum gradient but Stage I zoeae requires either a slightly larger gradient or they must increase their descent rate by active swimming to prevent sensory adaptation from occurring before detection.

A further consideration is the functional significance of the asymmetrical responses to salinity change. They were extremely sensitive to rates of salinity increase but did not display the expected response to a salinity decrease. It is unlikely that the response to salinity increase is used to avoid adverse high salinity conditions. Larvae can usually develop in the highest salinities available in their environment. For example, *R. harrisii* larvae can complete development in salinities as high as 40 ppt (Costlow *et al.*, 1966). The real tolerance problem is to low salinity water (Forward *et al.*, 1982).

With the present study it is possible to evaluate similarities in responses of *R. harrisii* zoeae to rates of change in salinity, light (Forward, 1985), and pressure (Forward and Wellins, 1989). Upon descending in the water column salinity increases in a stratified system, light intensity decreases and hydrostatic pressure increases. At rates of change that are within the range a larvae can encounter while descending, each of these environmental changes induces negative geotaxis and/or an activity increase that results in an ascent.

In contrast, the opposite environmental changes upon

an ascent produce weak responses at best. The present study indicated larvae do not descend in response to rates of salinity decreases they are likely to encounter in a salinity gradient. Larvae are unresponsive to rates of increase in light intensity that could be encountered underwater (Forward, 1985). In darkness, a positive geotaxis occurs to a pressure decrease but the threshold rate is much higher than that for a pressure increase (Forward and Wellins, 1989). Thus these asymmetrical responses of *R. harrisii* zoeae to environmental factors keep larvae up in the water column and reduce the likelihood that they will encounter the bottom and the associated benthic predators.

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